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Research Protocol

Surgery Section

Effect of Chemotherapy on the Coagulation Profile in Breast Cancer Patients: A Research Protocol

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ABSTRACT

Introduction: Thrombosis is a major cause of death for individuals with cancer. Clotting problems are common complications for cancer patients, often manifesting as Deep Vein Thrombosis (DVT), Pulmonary Embolism (PE) and Disseminated Intravascular Coagulation (DIC).

Need of the study: The mechanism of thrombogenesis is complex and is related to tumour physiology as well as the host's physiological response to the tumour. Understanding the correlation between cancer and coagulation profiles could facilitate the development of treatment modalities aimed at addressing both cancer progression and thrombotic complications in cancer patients. Moreover, it can contribute to attenuating cancer progression.

Aim: To determine the effect of chemotherapy on the coagulation profile in breast cancer patients.

Materials and Methods: A prospective observational study will be conducted involving 44 female patients aged 18 years and older who are admitted to the Inpatient Department of Oncology at AVBR Hospital in Sawangi, Wardha, Maharashtra, India from March 2023 to March 2025. Patients diagnosed with breast cancer will be included in the study after providing informed written consent. Demographic data will be collected and pre- and postchemotherapy investigations—including Complete Blood Count (CBC), Coagulation Profile and D-dimer—will be assessed over six cycles, with intervals determined by the patient's disease progression. Statistical analysis will utilise Analysis of Variance (ANOVA) to compare coagulation parameters {e.g., Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), International Normalised Ratio (INR), D-dimer} across different chemotherapy cycles. A t-test will be employed to compare these parameters between the two groups, while the Chi-square test will assess associations between clinicopathological staging and coagulopathy. The significance level for all tests will be set at p-value <0.05.

Keywords: D-dimers, Mortality, Prothrombin time, Thrombosis, Tumour

INTRODUCTION

Armand Trousseau, a French physician, was the first to establish the association between malignancies and thrombosis [1]. Coagulation dysfunction is one of the predominant complications in carcinoma patients, typically manifesting as DVT, PE and DIC [2]. There is an established relationship between coagulopathy and cancer. The mechanism of thrombosis is complex, with factors such as tumour physiology, primary tumour site, tumour volume, tumour stage, host physiological response to the tumour, tumour neoangiogenesis, type of therapeutic intervention used and the presence of metastasis all contributing to coagulation disorders in individuals suffering from cancer [3].

The tumour stage and primary site are the two major predictive factors for the risk of thrombosis [4]. High tumour volume also acts as a key risk factor for DVT, intravascular DIC and abnormalities in the clotting system. Tumour cells of different origins express varying procoagulant profiles and the clotting system can be triggered to different extents according to the type and stage of the malignant disease [2,3]. Multiple factors, such as decreased levels of coagulation inhibitors, the presence of antiphospholipid antibodies, tumour-derived Tissue Factor (TF), impaired fibrinolysis and acquired activated protein C resistance, contribute to the hypercoagulable state among cancer patients [4,5]. Tumour neoangiogenesis, along with impaired organ function, also leads to the activation of coagulation and fibrinolysis in cancer patients. It is a well-known fact that cancer progression and metastatic spread worsen a patient's hypercoagulable state, supporting the concept of a close relationship between tumour burden and haemostatic abnormalities [6].

Blood fluidity is maintained by the dynamic balance between the coagulation and fibrinolysis systems [7]. In the fibrinolytic system, under the action of plasmin, cross-linked fibrin is degraded, resulting in Fibrin Degradation Products (FDPs), part of which is D-dimer. The assessment of D-dimer plasma levels serves as an early diagnostic tool for identifying fibrinogenesis. Elevated concentrations of D-dimer signify an active coagulation cascade and subsequent fibrinolysis, indicative of thrombotic predisposition or occurrence [8]. In individuals with breast cancer, there is a notable increase in plasma D-dimer concentrations, as well as elevated levels of fibrinogen. Additionally, cancer patients often exhibit decreased Antithrombin III (AT III) activity, resulting in shortened aPTT and PT values, along with an increase in PT percentage (PT%) and a decrease in INR values [9]. Chemotherapy-induced coagulopathy is multifactorial and can affect different levels of the coagulation pathway. It may be due to low platelet counts resulting from decreased thrombopoiesis, endothelial injury due to cancer and chemotherapy and/or Thrombotic Microangiopathy (TMA) [10].

Chemotherapy-Induced Thrombocytopenia (CIT) is a common challenge in cancer management. Most chemotherapy protocols impact the synthesis of platelets, which frequently emerges as a primary dose-limiting adverse reaction [10]. Each chemotherapy agent differs in how it causes thrombocytopenia: alkylating agents affect stem cells, cyclophosphamide affects later megakaryocyte progenitors, bortezomib prevents platelet release from megakaryocytes and some treatments promote platelet apoptosis [11]. It often requires dose adjustments, treatment delays, or rarely, treatment discontinuation, thus negatively impacting treatment outcomes and putting patients at risk for bleeding complications [10]. Thrombopoietin is the main regulator of platelet

production. Chemotherapy dose reduction and platelet transfusions remain the major treatments for affected patients.

Anticancer chemotherapy may affect liver function and decrease the synthesis of both procoagulation and anticoagulation factors [11]. Though the side-effects associated with chemotherapy are typically transient, endothelial injury may persist for extended periods following anticancer treatment. TMA is a complication that may arise directly from certain malignancies, but more commonly results from the administration of anticancer therapy [12]. Injury to endothelial cells caused by chemotherapeutic agents may lead to a loss of antithrombotic properties and it is suggested that this may play a role in the increased risk of venous thrombosis [11,13]. The incidence of cancer drug-induced TMA during the last few decades is over 15%, mainly due to the introduction of anti-Vascular Endothelial Growth Factor (VEGF) agents [14]. Therefore, the present study aims to evaluate the effect of the chemotherapy regimen on the coagulation profile of breast cancer patients.

Primary objectives:

1. To determine the effect of chemotherapy on breast cancer in terms of changes in the blood morphological picture CBC and Peripheral Smear (PS).
2. To estimate the degree of derangement in PT, APTT, INR, D-Dimer levels, Bleeding Time (BT) and Clotting Time (CT).

Secondary objectives:

1. To assess the demographic profile of breast cancer patients.
2. To determine the association between the clinicopathological staging of breast cancer and coagulopathy.

Null hypothesis: There will be no significant effect of chemotherapy on the coagulation profile in breast cancer patients.

Alternate hypothesis: There will be a significant effect of chemotherapy on the coagulation profile in breast cancer patients.

REVIEW OF LITERATURE

TMA is a complication that can arise directly from certain malignancies but more commonly results from anticancer therapy. Previous literature has explored the connections between chemotherapy-induced coagulopathy and cancer-related coagulopathy; however, it has not been thoroughly examined the relationship among all three variables: cancer, chemotherapy and coagulopathy.

The purpose of this study will be to determine whether there is a significant relationship between chemotherapy and the coagulation profile in breast cancer patients and to better understand the extent of this relationship. By monitoring coagulation parameters and implementing appropriate interventions, it may be possible to minimise chemotherapy-induced coagulopathy.

Several studies have examined the relationship between coagulopathy and chemotherapy in breast cancer patients. According to research by Stoencheva S et al., there was a significant increase in D-dimer levels among breast cancer patients compared to a control group. Additionally, there was a shortening of aPTT and PT, with an increase in PT percentage and a decrease in INR values [15].

One study indicated that the hypercoagulable state was influenced not only by clinical staging (p-value <0.0001) but also by the site of metastasis (p-value <0.0001 for bone versus lung). In a group with positive D-dimer results, higher fibrinogen levels, prolonged aPTT and PT showed significant interactions with the disease's clinical stage (p-value <0.05). When comparing positive D-dimer groups with and without thrombus, there was a statistically significant difference in White Blood Cell (WBC) and D-dimer levels (p-value <0.05), although no such correlation was found with Red Blood Cell (RBC) and platelet counts. Furthermore, the hypercoagulable plasma profile in cancer patients showed improvement 2-3 weeks after chemotherapy (p-value <0.05 for the first six cycles) [4].

Kuter DJ proposed that the incidence of CIT was highest among regimens based on gemcitabine and platinum. Their study indicated that different chemotherapy agents impact thrombocytopenia through distinct mechanisms: alkylating agents affect stem cells, while cyclophosphamide targets later megakaryocyte progenitors [10].

MATERIALS AND METHODS

A prospective observational study will be conducted at Acharya Vinoba Bhave Rural Hospital in central India from March 2023 to March 2025. The Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research (Deemed to be University) has approved the study with the reference number DMIHER(DU)/IEC/2023/958. Before conducting the study, participants will be explained the study's goals and methods in their native language and asked to sign a written informed consent form.

Inclusion criteria: All admitted patients with histopathologically proven and confirmed diagnoses of breast cancer who are above 18 years of age will be included in the study.

Exclusion criteria:

- Patients who are discharged before completion of the treatment or transferred to another hospital.
- Patients on anticoagulants or antiplatelet drugs (such as heparin, aspirin, or haemocoagulase).
- Patients with a previous history of coagulopathy (DVT, PE).
- Patients with severe liver diseases.
- Patients with coagulation disorders (haemophilia/vitamin K disorders).
- Patients who have not given the consent for the study.

Sample size calculation: The sample size is calculated based on the difference in mean values for the variable PT-INR between pre and postchemotherapy. Primary Variable: coagulation profile (PT in sec);

- Mean coagulation profile (PT) (pre)=11.5;
- Mean coagulation profile (PT) (after 8 days)=12.1;
- Difference in mean (δ)=0.6 [16];
- Considering normal Std Deviation=1;

Formula using mean difference:

$$n1=n2=\frac{(Z\alpha+Z\beta)^2\,\sigma^2}{(\delta)^2}$$

$Z\alpha$=1.96 at 5% error and CI at 95%

$Z\beta$=0.84=Power at 80%.

n=44.

A total of 44 patients above 18 years of age will be included in the study.

Sample collection: Samples will be collected using a 21-gauge needle into vacutainer tubes containing 0.109M citrate (9:1 vol/vol; Becton Dickinson) and Ethylenediamine Tetraacetic Acid (EDTA) tubes to conduct the CBC and PS. EDTA acts as a chelating agent that binds calcium, which is necessary for enzyme reactions in the coagulation cascade.

Demographic details of the patients will be obtained (age, sex, inpatient department number, address, weight/height of the patient, Body Mass Index (BMI), type of chemotherapy and cumulative doses of drugs). A total of 6 mL of blood will be taken from each patient during each cycle of chemotherapy, before the administration of chemotherapy and 24 hours after the completion of chemotherapy. The patient will be followed-up with six cycles of chemotherapy. A baseline laboratory investigation, including CBC with PS, BT, CT, PT, aPTT, INR and D-dimer, will be done before starting chemotherapy. A second sample will be taken 24 hours after the administration of chemotherapy.

CBC principal: A suspension of blood cells is passed through a small orifice simultaneously with an electric current. The individual blood cells passing through the orifice introduce an impedance change in the orifice determined by the size of the cell. The system counts the individual cells and provides cell size distribution. The number of cells counted per sample is approximately 100 times greater than the usual microscope count, reducing the statistical error factor by approximately 10 times. PS will be performed by a senior pathologist.

Suspension citrate chelates free calcium ions, preventing them from forming a complex with TF and coagulation factor VIIa, which promotes the activation of coagulation factor X. This inhibits the extrinsic initiation of the coagulation cascade [17].

Coagulation profile principle: The analyser detects transmitted light. The light from the source is spectrally split into five beams of wavelengths 340, 405, 575, 660 and 800 nm, which are shown on the reagent-sample mixture. The transmitted light of each wavelength is detected every 0.1 seconds. The transmitted light is then converted into an electrical signal and the coagulation time or concentration is calculated by a microprocessor. This is called a multi-wavelength detection system as light of five different wavelengths is used for analysis. A 340 nm interference filter, which is in the ultraviolet range, is provided as a standard component [18].

D-dimer principle: It is detected by an automated turbidimetric immunoassay using monoclonal antibodies bound to latex beads [19].

Outcome parameters: PT, aPTT, INR, D-Dimer levels, BT, CT, CBC and PS.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) version 25.0 will be used for statistical analysis. ANOVA will be employed to compare the means of various groups to determine if there are statistically significant differences in the coagulation profile parameters across different chemotherapy cycles. The unpaired t-test will be utilised to compare the means of two groups. To analyse categorical variables, the Chi-square test will be applied, potentially examining associations between clinicopathological staging and the presence of coagulopathy or other binary outcomes. The level of statistical significance for all these tests will be set at a p-value <0.05.

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